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TOXICITY OF A PHENYL PYRAZOLE INSECTICIDE, FIPRONIL, TO MOSQUITO AND CHIRONOMID MIDGE LARVAE IN THE LABORATORY

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ABSTRACT. Toxicity of a phenyl pyrazole insecticide, fipronil, to 4th-instar larvae of 6 species of colonized mosquitoes (*Aedes aegypti, Ae. albopictus, Ae. taeniorhynchus, Anopheles quadrimaculatus, Culex nigripalpus,* and *Cx. quinquefasciatus*) and 2 species of field-collected chironomid midges (*Chironomus crassicaudatus* and *Glyptotendipes paripes*) was evaluated in the laboratory. All mosquito species were highly susceptible with 48-h median lethal concentration (LC_{50}) values ranging from 0.00043 ppm (*Ae. taeniorhynchus* and *An. quadrimaculatus*) to 0.023 ppm (*Ae. albopictus*). *Chironomus crassicaudatus* and *G. paripes* also were extremely susceptible (48-h LC_{50} of both species: 0.00042 ppm) to fipronil. Larval mortality checks of *Ae. taeniorhynchus, Cx. nigripalpus,* and *G. paripes* at 24 h and again at 48 h posttreatment revealed delayed activity of this compound against these species. First-instar larvae of *Ae. albopictus* and *Cx. quinquefasciatus* were significantly ($P < 0.01$) more susceptible to fipronil than the 4th-instar larvae of these mosquito species.

KEY WORDS *Aedes* spp., *Anopheles quadrimaculatus, Culex* spp., Chironomidae, *Chironomus crassicaudatus, Glyptotendipes paripes,* fipronil, laboratory bioassays

Fipronil ((±)-5-amino-1-(2,6-dichloro-α-α-α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile) belongs to a new class of insecticides known as phenyl pyrazoles. Fipronil was discovered by Rhone-Poulenc Agro in 1987 at Ongar, United Kingdom (Colliot et al. 1992). Although fipronil affects the central nervous system (CNS) of insects, its mode of action is unique in that it interferes with the passage of chloride ions through the gamma-aminobutyric acid (GABA)-regulated chloride channel (Cole et al. 1993), thereby disrupting CNS activity, and at sufficient doses, causing death. At present, fipronil is registered for agricultural applications against both piercing–sucking and chewing phytophagous insects in more than 30 countries. In the USA, fipronil is registered for use on golf courses and against fleas and ticks on cats and dogs (Anonymous 1996a). Because of fipronil's broad spectrum of activity encompassing a wide variety of pests, this compound is currently being investigated and developed for public health use purposes. Reported here is the activity of fipronil in the laboratory against larvae of medically and/or economically important aquatic insects, mosquitoes, and chironomid midges.

Technical grade fipronil (97.1%) in 6–7 serial dilutions in acetone was utilized in these evaluations. For mosquito bioassays, 4th-instar larvae of *Aedes aegypti* (Linn.), *Aedes albopictus* (Skuse), *Aedes taeniorhynchus* (Wiedemann), *Anopheles quadrimaculatus* Say, *Culex nigripalpus* Theobald, and *Culex quinquefasciatus* Say were utilized. First-instar larvae of *Ae. albopictus* and *Cx. quinquefasciatus* also were used to determine any susceptibility differences with their respective 4th-instar larvae. Larvae of tested mosquitoes were obtained from colonies maintained at the University of Florida's Florida Medical Entomology Laboratory (FMEL), Vero Beach, FL. For chironomid bioassays, 4th-instar larvae of *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards were collected from Lakes Jessup and Monroe, in central Florida.

The procedures for mosquito and midge bioassays in this study were similar to those of Mulla et al. (1966) and Mulla and Khasawinah (1969). For mosquito bioassays, 20 larvae were placed in a 120-ml disposable cup containing 100 ml of tap water. Six or 7 different concentrations of fipronil were tested against each mosquito species each time. Each concentration was replicated 3 times and 3 untreated cups were used as controls. Larval mortality in each cup was checked at 24 h and again at 48 h posttreatment for the first few tests. Because of some mortalities occurring beyond 24 h posttreatment, the larval mortality of each mosquito species was scored at 48 h posttreatment. One milliliter of 1% beef liver plus yeast (1:1) was added daily to each cup. The experimental design for bioassays of chironomid larvae and the time interval of mortality checks were the same as used for mosquito larvae, except that 5 g of sterilized fine sand (as substrate to prevent larval cannibalism) was added to each midge bioassay cup to which 0.02 g of ground dog food (Dog Biscuits, Publix Super Markets, Inc., Lakeland, FL) suspended in 1 ml of distilled water also was added daily. A 14-h light, 10-h dark photoperiod and 26 ± 2°C were maintained in the evaluation room during the tests. Midge or mosquito larval mortality in treated cups was corrected for any larval mortality in corre-

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Table 1. Susceptibility of laboratory-reared 4th-instar mosquito larvae, and field-collected 4th-instar chironomid midge larvae to fipronil in the laboratory.

Species	48-h lethal concentration (ppm)[1]		
	LC_{50}	95% CL	Slope
	Mosquitoes		
Aedes aegypti	0.00154	0.00143–0.00165	4.28
Aedes albopictus	0.023	0.015–0.032	1.95
Aedes taeniorhynchus	0.00043	0.00034–0.00050	4.19
Anopheles quadrimaculatus	0.00043	0.00009–0.00081	2.51
Culex nigripalpus	0.00087	0.00042–0.00147	3.16
Culex quinqefasciatus	0.0073	0.0069–0.0077	5.46
	Midges		
Chironomus crassicaudatus	0.00042	0.00032–0.00052	2.23
Glyptotendipes paripes	0.00042	0.00016–0.00080	2.18

[1] LC_{50}, median lethal concentration; CL, confidence limits.

sponding controls and the data were subjected to a log-dose-probit regression analysis (U.S. Environmental Protection Agency 1994) to estimate fipronil dosage response of exposed mosquito and midge larvae. In a couple of instances where a lower level of larval mortality was observed at a higher serial dose, the data were not included in the analysis because in these replicates the active ingredient perhaps settled out of suspension.

Mosquito and midge larval susceptibility data to fipronil are presented in Table 1. All mosquito species were highly susceptible to fipronil, with median lethal concentration (LC_{50}) values ranging from 0.00043 ppm (*Ae. taeniorhynchus* and *An. quadrimaculatus*) to 0.023 ppm (*Ae. albopictus*). Among the 3 *Aedes* species, *Ae. taeniorhynchus* was the most susceptible and *Ae. albopictus* the least susceptible (Table 1). Fipronil was highly effective against *An. quadrimaculatus*. Fipronil also was highly toxic to both *Culex* species; *Cx. nigripalpus* (LC_{50} = 0.00087 ppm) was 9-fold more susceptible than *Cx. quinquefasciatus* (LC_{50} = 0.0073 ppm). Among the mosquitoes tested, there was a 53-fold susceptibility difference between the least susceptible (*Ae. albopictus*) and the most susceptible (*Ae. taeniorhynchus* and *An. quadrimaculatus*) species. Both chironomid species, *C. crassicaudatus* and *G. paripes*, coincidentally had the same LC_{50} value of 0.00042 ppm, indicating the extremely good activity of fipronil against these midges. These midge species in terms of susceptibility to fipronil ranked at the same level of the most susceptible mosquitoes, *Ae. taeniorhynchus* and *An. quadrimaculatus*.

Mortality of *Ae. taeniorhynchus*, *Cx. nigripalpus*, and *G. paripes* larvae occurring beyond 24 h posttreatment is evident from Table 2. The larval mortality (LC_{50}) differences between 24 h and 48 h posttreatment for *Ae. taeniorhynchus* and *Cx. nigripalpus* were 3.25- and 1.61-fold, respectively. *Glyptotendipes paripes* larvae also were 2.16-fold more susceptible to fipronil at 48 h posttreatment compared to their susceptibility (LC_{50}) at 24 h posttreatment.

Susceptibilities of 1st- and 4th-instar larvae of *Ae. albopictus* and *Cx. quinquefasciatus* to fipronil are presented in Table 3. First-instar larvae of both mosquitoes were more susceptible than the 4th-instar larvae (2.8-fold and 1.6-fold difference for *Ae. albopictus* and *Cx. quinquefasciatus*, respectively). Two-way analysis of variance revealed significant ($P < 0.01$) susceptibility difference between the 2

Table 2. Mortality response of laboratory-reared 4th-instar larvae of *Aedes taeniorhynchus* and *Culex nigripalpus* mosquitoes, and field-collected 4th-instar larvae of *Glyptotendipes paripes* midge to fipronil at 24 h and 48 h posttreatment in the laboratory.[1]

Time (h)	LC_{50} (ppm)	95% CL	Slope
	Aedes taeniorhynchus		
24	0.0014	0.00119–0.00163	5.51
48	0.00043	0.00034–0.0005	4.19
	Culex nigripalpus		
24	0.0014	0.00134–0.00152	3.38
48	0.00087	0.00042–0.00147	3.16
	Glyptotendipes paripes		
24	0.00091	0.00055–0.00141	2.04
48	0.00042	0.00016–0.00080	2.18

[1] LC_{50}, median lethal concentration; CL, confidence limits.

Table 3. Susceptibility of laboratory-reared 1st- and 4th-instar larvae of *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes to fipronil at 48 h posttreatment in the laboratory.[1]

Instar	LC_{50} (ppm)	95% CL	Slope
	Aedes albopictus		
First	0.0081	0.0071–0.0090	3.80
Fourth	0.023	0.015–0.032	1.95
	Culex quinquefasciatus		
First	0.0046	0.00004–0.0087	2.35
Fourth	0.0073	0.0069–0.0077	5.46

[1] LC_{50}, median lethal concentration; CL, confidence limits.

larval instars of both species. No significant interaction of dosage with larval instars was found.

No published laboratory data are available on the activity of fipronil against larval mosquitoes and chironomids for comparison purposes. However, toxicity of fipronil to various mosquito species in our study was generally in the same range as the most effective insect growth regulators (IGRs). These include: diflubenzuron, pyriproxyfen, and UC-84572, and abamectin (MK-936) tested earlier against the same laboratory-colonized mosquito species, *Ae. aegypti, Ae. albopictus, Ae. taeniorhynchus, An. quadrimaculatus, Cx. nigripalpus,* and *Cx. quinquefasciatus* at FMEL. The LC_{50} values for abamectin (0.0007–0.0077 ppm), and the IGRs had ranged from 0.00011 to 0.002 ppm against these mosquito species (Ali and Nayar 1985, 1987; Ali et al. 1995). The present chironomid susceptibility data on fipronil (LC_{50} = 0.00042 ppm) also are compatible with the most active IGRs, UC-84572, abamectin (MK-936), and some experimental pyrethroids such as FMC-45499 and FMC-52703 tested against field-collected *C. crassicaudatus* and *G. paripes* from central Florida. The LC_{50} range for these species was 0.00012–0.0026 ppm (Ali and Lord 1980, Ali 1981, Ali and Stanley 1981, Ali and Nayar 1987).

The superior biological activity of fipronil against larvae of the tested mosquito and midge species is evident from this study. At present, no independently published data are available showing the adverse effects of fipronil on nontarget aquatic organisms. However, in-house laboratory studies by Rhone-Poulenc (Anonymous 1996b) on some aquatic organisms indicate 96-h LC_{50} values of 0.25 ppm (rainbow trout), 0.43 ppm (European carp), 0.085 ppm (bluegill sunfish), and 0.19 ppm (*Daphnia magna,* 48-h exposure). Thus, this insecticide seems to be relatively safe to these nontarget aquatic organisms. Development of fipronil for mosquito and midge control purposes is highly desirable because of its attributes of high levels of toxicity, novel mode of action suitable for targeting mosquito and midge species resistant to other insecticides, and perhaps relative safety to nontarget aquatic organisms. This new molecule will be a very useful addition to the rather dwindling arsenal of mosquito control in the USA; however, further investigations, especially field studies, on fipronil against mosquitoes, midges, and nontarget aquatic organisms are warranted to further confirm the target and nontarget effects of this new insecticide.

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